101. (Amended) A composition comprising transgenic totipotent bovine CICM cells of a CICM cell line that express a transgene, and also comprising cells of the same CICM cell line that do not express the transgene,

wherein the CICMs of the CICM cell line stably exhibit the following properties:

(a) small cytoplasmic/nuclear volume ratio ranging from 10/90 to 50/50;

(b) observable cytoplasmic vesicles; and

(c) individual cells ranging from about 10 μm to 20 μm in diameter.

102. (Amended) A composition comprising transgenic totipotent bovine CICM cells of a CICM cell line that express a transgene, and also comprising cells of the same CICM cell line that do not express the transgene,

wherein the CICMs of the CICM cell line are alkaline protease positive and cytokeratin 18 negative.

REMARKS

This Reply is responsive to the Office Action dated August 12, 2002. Claims 106-120 are canceled, and claims 91, 101, 102 are amended. Entry of the foregoing and reconsideration on the merits is respectfully requested.

Regarding the Objections to Claims 91, 101, and 102:

Claim 91 is amended to recite the term "cultured inner cell mass" in order to identify the abbreviation "CICM," support for which is found on page 15 (mid-page); and claims 101 and 102 are amended by re-writing them as independent claims. These amendments are believed to overcome the grounds for objection stated on pages 4 and 5 of the office action.

Regarding the Rejection of Claims 91-105 under 35 U.S.C. § 112, 1st Paragraph:

Claims 91-105 are rejected as failing to comply with the "written description requirement" of 35 U.S.C. § 112, 1st paragraph, by claiming subject matter that is not described in the specification. The Applicants respectfully traverse the rejection.

Claim 91 is drawn to:

> "A composition comprising transgenic totipotent bovine cultured inner cell mass (CICM) cells of a CICM cell line that express a transgene, and also comprising cells of the same CICM cell line that do not express the transgene."

The claimed composition is an important element of the invention disclosed by the present application, and is described numerous times in the specification. For example, the method disclosed at the bottom of page 11 and the top of page 12 describes preparing and culturing CICM cells in vitro (page 11), inserting heterologous DNA into the CICM cells, and selecting for transgenic cells (page 12). A similar method in which heterologous DNA is inserted into CICM cells in vitro and transgenic cells are selected is described on page 13 (mid-page). On page 19, the specification expressly states that "the present invention is also directed to the introduction of heterologous DNA into cultured inner cell mass (CICM) cells developed from novel ICM cells, [and] the subsequent selection for transgenic CICM cells ...." The specification teaches inserting into CICM cells heterologous DNA that includes a selectable marker in addition to the gene of interest, in order to identify and select cells transformed with the gene of interest (page 30, mid-page). Seven different, well known selectable marker genes that can be used in this manner are disclosed on page 30 (Neo, HPH, etc.). One skilled in the art would know that these marker genes permit selection by conferring resistance to a cytotoxic selective agent on a cell in which they are expressed (see J. Sambrook et al., Molecular Cloning, A Laboratory Manual, 1989, Cold Spring Harbor Press, Cold Spring Harbor, NY, pages 16.8-16.14, attached). For example, cells that do not express the Neo gene can be killed by the selective agent G418, whereas transgenic cells transformed to express the Neo gene survive and grow in the presence of G418. The specification also describes introducing into CICM cells heterologous DNA that includes a

marker gene such as a gene encoding beta-galactosidase, which permits visual identification of transgenic CICM cells in which the transgene is expressed (see sentence bridging pages 29-30, for example). The specification further describes a method for identifying and selecting transgenic CICM cells by inserting heterologous DNA that includes a differentiation-inhibiting (DI) gene into CICM cells, and culturing the cells under conditions that result in differentiation of cells in which the transgene is not expressed (page 31, and Example 4). Examples of the claimed composition in which bovine CICM cells were transfected with heterologous DNA comprising a transgene encoding beta-galactosidase and/or neomycin phosphotransferase are described in Examples 2 and 3 (pp. 38-42). When the composition of transfected CICM cells described in Example 3 was cultured in the presence of G418, the transgenic CICM cells expressing the Neo gene survived, and the other CICM cells died (p. 41). Each of these disclosed methods, as well as the actual working examples described in the specification, results in production of the claimed composition. As recited by claim 91, the claimed composition comprises totipotent bovine CICM cells of a CICM cell line that are present in two distinct forms - (1) as transgenic CICM cells that express a transgene, and (2) as CICM cells of the same CICM cell line that do not express the transgene. The transgenic CICM cells of the first group are produced by introducing heterologous DNA that includes a marker gene that is expressed in the cells, as described in the specification. The CICM cells of the second group are cells that do not express the transgene. The specification expressly describes making the claimed composition, and then selecting the transgene-expressing CICM cells from the CICM cells that do not express the transgene. Persons skilled in the art know that cells that are exposed to heterologous DNA comprising a transgene may fail to express the transgene, either because the heterologous DNA fails to enter a cell, or because the DNA enters a cell but is not expressed. For the purposes of the claimed invention, it is immaterial whether a CICM cell in the claimed composition is unselected because it did not take up the heterologous DNA, or because it took up the DNA but failed to express it. Accordingly, the only distinction made between the

two type of CICM cells in the claimed compositoion is between those transgenic CICM cells that express the transgene, and those CICM cells that do not express the transgene. This is the same distinction described in the specification.

As discussed above, the claimed composition is literally described in the specification. Methods for using the claimed composition are also described. Accordingly, the Applicants respectfully request withdrawal of the rejection of the claims under 35 U.S.C. § 112, 1st paragraph, for claiming subject matter that is not described in the specification.

Rejections of claims under 35 U.S.C. §112, second paragraph:

Claims 91-105 were rejected under 35 U.S.C. §112, second paragraph as being indefinite, because of ambiguity in reciting cells of a single cell line that differ in their ability to express a transgene as recited in claim 91. The Applicants respectfully submit that the meaning of claim 91 is clear to persons skilled in the art. The premise of the claimed invention is that the claimed composition contains two classes of CICM cells - those that express a transgene (and so are transgenic), and those that do not express the transgene. As discussed above, persons skilled in the art would recognize that the cells that do not express the transgene may fail to do so for any of several reasons; e.g., they may have taken up the DNA, but is failed to integrate, and was lost during subsequent passaging, or the DNA may have integrated into heterochromatin. Alternatively, the cells may never have taken up the heterologous DNA in the first place. As pointed out above, it is immaterial why the non-expressing cells do not express the transgene. The specification teaches that the claimed composition is useful because it can be subjected to selection to select the CICM cells expressing the transgene from those that do not. The Applicants respectfully request withdrawal of the rejection of the claims under 35 U.S.C. § 112, 2nd paragraph, as being indefinite.

Claims 101 and 102 were rejected under 35 U.S.C. § 112, 2nd paragraph, as being indefinite because they recite characteristics that are regarded as inherent to the CICM cells recited in independent claim 91. Claims 101 and 102 are amended by re-writing them as independent claims. Withdrawal of the rejection of claims 101 and 102 under 35 U.S.C. § 112, 2nd paragraph, is respectfully requested.

Rejections of claims under 35 U.S.C. §102(e)

Claims 91-95 and 101-105 were rejected under 35 U.S.C. §102(e) as being unpatentable over Sims (U.S. Patent No. 6,107,543). The Applicants respectfully traverse the rejection. The claimed composition comprises totipotent bovine CICM cells of a CICM cell line that are present in two distinct forms - (1) as transgenic CICM cells that express a transgene, and (2) as CICM cells of the same CICM cell line that do not express the transgene. Such a composition is not disclosed by the Sims patent. The Sims patent alleges that bovine CICM cells produced by its method can be transfected and selected in vitro to produce totipotent, genetically modified ES cells, but it does not show that this is possible. As discussed in our response to the previous office action, it was well known by persons skilled in the art at the time the invention was made that the totipotency of ES cells is an unstable characteristic, and that genetic manipulation of ES cells in vitro can cause the ES cells to lose their totipotency, unless the cells are handled under the proper conditions. The present application has demonstrated conditions suitable for preparing totipotent, genetically modified bovine CICM cells. The Sims patent has not. Moreover, the Sims patent expressly states that bovine ES cells cultured on feeder cells differentiate into epithelial cells, and teaches culturing the cells in suspension to prevent this from happening (col. 14, lines 44-56). In contrast, the present application discloses a method for producing totipotent, genetically modified bovine CICM cells in which the CICM cells are cultured on a feeder layer. It is well known that cells growing in suspension differ physiologically and structurally from cells growing on a monolayer. Therefore, there is no scientific basis for assuming that cells

produced by the methods described in the Sims patent will retain their totipotency after genetic manipulation in the same manner as the cells of the claimed composition.

The office action suggests that the claimed cells are not totipotent, because the developing fetuses did not develop to full term and live birth. The Applicants respectfully disagree with the interpretation of totipotency implied by this argument. Totipotency refers to the ability of a stem cell to differentiate into different type of cells, not to the ability of cells of an embryo to develop into an animal of a given stage of development. The specification demonstrates that embryos containing the claimed cells developed into normal fetuses, and that the transgenic CICM cells contributed to a wide variety of different cell types in the fetuses, including primordial germ cells (page 44). Persons skilled in the art recognize that the development of viable transgenic animals from normal fetuses is a "numbers game," and would reasonably regard the production of normal transgenic fetuses by the claimed method as evidence that normal transgenic live animals could also be produced. The fact that the claimed method generates normal fetuses with transgenic primordial germ cells is evidence that it can produce animals that can be used to breed transgenic progeny. The importance of being able to genetically modify embryo-derived cells such as ES or CICM cells and use them to generate transgenic animals cannot be overstated.

Given the differences between the methodology of the Sims patent and that of the present application, given the recognized difficulties associated with genetically modifying ES cells without loss of totipotency, especially in a species such as bovine, in which the art is undeveloped and responses are unpredictable, and given that the Sims patent only talks about genetically modifying stem cells, but does not actually disclose the claimed composition, it is improper to reject the claimed composition over the limited disclosure of the Sims patent.

Rejections of the claims under 35 U.S.C. §103(a)

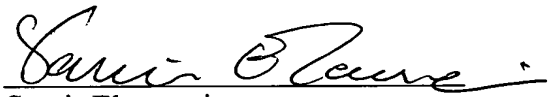
Claims 91-95 and 98-105 were rejected under 35 U.S.C. §103(a) as being unpatentable over Sims et al. (1993), Deboer et al., and Stewart et al. Like the Sims patent, the published article by Sims et al. cited as prior art in this rejection teaches culturing bovine ES cells in suspension to prevent their differentiation; and like the Sims patent, the article by Sims et al. only proposes genetically modifying the ES cells - it does not actually show that this can be done with retention of totipotency. This deficiency is not remedied by the Deboer et al. or Stewart et al. references. From studies performed with murine ES cells, it is well known in the art that ES cells are very sensitive to the conditions under which they are cultured, and that if these conditions are not strictly controlled, the cells will lose their totipotency when attempts are made to genetically manipulate them. It is reasonable to assume, until it is shown otherwise, that the same is also true for other species, including bovine. As discussed in the reply to the 102 rejection, the differences between the method for producing totipotent ES cells described by Sims et al. and the method for producing totiopotent CICM cells of the present invention are significant. There is simply no scientific basis for assuming that since the Applicants' method was successful in producing transgenic, totipotent bovine CICM cells, the method of Sims et al. would also work to produce transgenic, totipotent bovine ES cells. At the time the invention was made, there was widespread recognition of the commercial importance of being able to genetically modifying totipotent ES cells and use them to produce transgenic cows and pigs. That transgenic, totipotent bovine CICM cells were not disclosed prior to Applicants' invention is additional evidence of the non-obviousness of the invention. Withdrawal of the rejection is requested.

The above amendment and remarks are fully responsive to the Office Action. If there are any issues remaining that need to be resolved, the Examiner is respectfully requested to contact the undersigned so that allowance of this application can be expedited.

Respectfully submitted,

PILLSBURY WINTHROP LLP

Date: February 12, 2003

By: 
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APPENDIX

Claims 91, 101, and 103 are amended as shown below:

91. (Amended) A composition comprising transgenic totipotent bovine cultured inner cell mass (CICM) cells of a CICM cell line that express a transgene, and also comprising cells of the same CICM cell line that do not express the transgene.

101. (Amended) [The composition of claim 91] A composition comprising transgenic totipotent bovine CICM cells of a CICM cell line that express a transgene, and also comprising cells of the same CICM cell line that do not express the transgene,

wherein the CICMs of the CICM cell line stably exhibit the following properties:

(a) small cytoplasmic/nuclear volume ratio ranging from 10/90 to 50/50;

(b) observable cytoplasmic vesicles; and

(c) individual cells ranging from about 10 μm to 20 μm in diameter.

102. (Amended) [The composition of claim 91] A composition comprising transgenic totipotent bovine CICM cells of a CICM cell line that express a transgene, and also comprising cells of the same CICM cell line that do not express the transgene,

wherein the CICMs of the CICM cell line are alkaline protease positive and cytokeratin 18 negative.